REMARKS

Reconsideration and allowance are respectfully requested. Applicants gratefully acknowledge the Examiner's withdrawal of a large number of the claim rejections raised in the previous Office Action.

Claims 1-10, 12-13, 15-18 and 20-46 are pending.

The amendments are fully supported by the original disclosure and, thus, no new matter has been added. If the Examiner should disagree, however, she is respectfully requested to point out the challenged limitation with particularity in the next Action so support may be cited in response.

Specification/Claim Objections

The specification was objected to by the Examiner. The brief description of the drawing is amended. This section finds basis in the specification at page 38, line 17, to page 39, line 6. It is apparent that the third column of each set in Figure 1 represents the result for intranasal immunization with *Lactobacillus plantarum* pLP503-TTFC as indicated in the brief description of the drawing and page 38, line 31, to page 39, line 6, of the specification. Accordingly, the brief description of the drawing does not add new matter.

Claims 3-6 and 9 were objected to by the Examiner. The terms "immunogenicity" and "immunity" were objected to with reference to inducing an immune response. Basis for the induction of immunogenicity or an immune response against a pathogenic micro-organism that the heterologous antigen is derived from is provided at page 12, lines 28-30, and page 7, line 30, to page 8, line 2, of the specification. Claims 3-6 and 9 as originally filed make clear that the result of inducing immunogenicity or an immune response against a heterologous antigen is to induce immunogenicity or an immune response against the pathogenic microorganism that the heterologous antigen derives from. Indeed, the general purpose of oral vaccination with a pathogenic antigen is to induce an immune response against the pathogen. *Mycobacterium tuberculosis* is italicized as suggested by the Examiner.

Withdrawal of the objections is requested.

35 U.S.C. 112 – Written Description

Claims 5 and 9 were rejected under Section 112, first paragraph, because it was alleged that they contain "subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." Applicants traverse.

Claims 5 and 9 are amended to replace "pathogenic organism" with --pathogenic microorganism--. The specification fully supports eliciting an immune response against a *pathogenic microorganism*. The terms "immunogenicity" and "immunity" are replaced by --immune response-- in the claims. Basis for the induction of immunogenicity or an immune response against a pathogenic microorganism that the heterologous antigen is derived from is provided at page 12, lines 28-30, and page 7, line 30, to page 8, line 2, of the specification.

Indeed, in situations where the heterologous antigen expressed by a vaccine of the invention is derived from a pathogenic microorganism then, by definition, because the oral vaccines of the invention give rise to an immune response against the heterologous antigen, they will also give rise to an immune response against the pathogenic microorganism as it expresses the heterologous antigen. Claims 5 and 9 are supported by Applicants' disclosure as originally filed.

Applicants' specification plainly contemplates eliciting or inducing an immune response against pathogenic microorganisms such as those described at page 12, lines 24-27, and claim 3. In general, the purpose of vaccines is to induce such an immune response and it is also apparent from the various antigens mentioned at pages 15-17 of the specification that one of the objectives of Applicants' invention is to induce an immune response against pathogenic microorganisms.

Withdrawal of the written description rejection made under Section 112, first paragraph, is requested because the specification conveys to a person skilled in the art that Applicants were in possession of the claimed invention as of the filing date.

35 U.S.C. 112 – Definiteness

Claims 1-25 and 31-32 were rejected under Section 112, second paragraph, as being allegedly "indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." Applicants traverse.

Claim 5 is amended to refer to *Pneumocystis carnii* as suggested by the Examiner. As indicated in the Action, this is the pathogenic microorganism responsible for pneumonia and the art would recognize that the original reference to "*Pneumocystis pneumonia*" was to --*Pneumocystis carnii*-- as that is the pathogenic microorganism responsible for pneumonia. Hence no new matter is added by the amendment.

Claim 16 is amended to specify that the recombinant *Lactobacillus plantarum* is recombinant *Lactobacillus plantarum* 256. The amendment makes clear that there is only one *Lactobacillus plantarum* 256.

Claim 1 is amended to clarify that the immune response is elicited in the subject to whom the vaccine is administered. Thus, whom the immune response is raised in is clear. Claim 1 is also amended to replace "can elicit an immune response" with --elicits an immune response--. It is therefore clear that eliciting an immune response is not optional feature of the claimed invention.

Similarly, claims 9 and 18 are amended to specify that the vaccine --induces a protective immune response-- and that the bacterium --elicits an immune response--, respectively. Both claims therefore are clear that eliciting an immune response is not an optional feature of the claimed invention.

In order to facilitate prosecution, claims 3-5 are amended as suggested by the Examiner to replace the term "immunogenicity" with --an immune response--.

Claims 1-2 and 18 are amended to delete the term "capable" and to indicate that the *Lactobacillus plantarum* is one --which expresses-- the heterologous antigen. The claims therefore are clear that expression of the heterologous antigen is not an optional feature of the claimed invention.

Claim 18 is amended to delete the term "optionally" and the feature previously indicated as optional has been made the subject of new dependent claim 42.

The features indicated in claims 2-3, 7, 12-13 and 18 as optional, suitable, or preferable are made the subjects of new dependent claims 33-41. The related terms are deleted from the claims in question.

In order to facilitate prosecution, claim 14 is deleted which renders the rejection moot.

Claim 9 is amended to refer to --a pathogenic microorganism--.

In order to facilitate prosecution, claims 11 and 14 are deleted which renders the rejection moot.

Claim 20 is amended to clarify that it is directed to a *Lactobacillus plantarum* bacterium from a non-human food-stuff or one which is of non-human origin. Claim 20 therefore is clear that it is the origin of the bacterium is being referred to.

Claim 20 is amended to delete the phrase "which has been modified to express" and now specifies that the bacterium is one --expressing a heterologous antigen--. The claim no longer refers to modification.

Claim 21 is amended to delete reference to the bacterium being "a naturally occurring or unmodified *L. plantarum*" for consistency with claim 20.

Claim 24 is amended to remove the parentheses and to clarify that it is not an optional feature of the claimed invention.

Claims 5 and 9 are amended to reintroduce --pathogenic microorganism--. The language in claims 5 and 9 is therefore consistent with that employed in claims 3 and 4 where a "pathogenic microorganism" is also referred to. Claims 31 and 35 are also amended to refer to a --pathogenic microorganism-- for consistency.

The second reference to "*Clostridium perfringens*" is deleted from claim 5 so that the claim only refers to the pathogenic microorganism once.

Claim 13 is amended to delete the term "strain" and now refers to --the recombinant *Lactobacillus plantarum*-- to be consistent with claim 1.

Claim 13 is amended to clarify that the subject is vaccinated orally with the vaccine of claim 1. Persistence occurs in that vaccinated subject.

Claim 18 is amended to provide appropriate antecedent basis for bacterium.

In order to facilitate prosecution, claim 19 is deleted which renders the rejection moot.

Claim 22 is amended to replace the term "that" with --said-- as suggested by the Examiner.

Claim 23 is amended to refer to --The recombinant *Lactobacillus plantarum*-- as suggested by the Examiner.

Due to the amendments to claims 1 and 18, the rejections raised against claims 2-17, 19, 23, 25 and 31-32 are also addressed.

Applicants request withdrawal of the Section 112, second paragraph, rejection because the pending claims are clear and definite.

35 U.S.C. 102 – Novelty

A claim is anticipated only if each and every limitation as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil Co. of Calif.*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). The identical invention must be shown in as complete detail as is claimed. See *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

Claims 1-7 and 9-25 were rejected under Section 102(b) as allegedly anticipated by Pouwels *et al.* (J. Biotechnol. 44:183-192, 1996) as evidenced by Hoshino *et al.* or Virelizier, Naidu and Wells *et al.* Applicants traverse.

Applicants respectfully disagree with the rejection raised in the Office Action that Figure 4 of Pouwels *et al.* shows that *Lactobacillus plantarum* was orally administered to mice. As discussed below, it is not possible for a skilled person to ascertain from Figure 4 which *Lactobacillus* strain was administered by Pouwels *et al.* But from the results presented in Pouwels *et al.*, the most likely strain to have been administered would have been *Lactobacillus casei* (evidence for this is discussed below). There is therefore no demonstration that any of the *Lactobacillus plantarum* strains discussed in Pouwels *et al.* would be able to give rise to an immune response when administered orally.

As discussed in the response to the previous Office Action, though something may be "antigenic" (i.e., antibodies may potentially be raised against it), that is not the

same as something which is "immunogenic" (i.e., something which actually gives rise to an immune response when administered). Something may be antigenic (i.e., have the ability to be recognized by antibodies) but not be immunogenic (i.e. not actually give rise to an immune response when administered) because of the context or way in which is it administered. Thus, whilst the vast majority of protein sequences are antigenic, it is only when administered in the appropriate context that they are actually immunogenic and give rise to an immune response. This is a fundamental distinction which is understood in the field of vaccines.

A vaccine which is antigenic, but not immunogenic, is a failure because it will not elicit an immune response when administered. Thus, when the putatively vaccinated subject actually encounters the actual pathogenic microorganism that the vaccine was intended to protect against, no immune response will be induced against the pathogenic microorganism and no benefit will be derived from the vaccination.

Thus, although the strain described in Pouwels *et al.* may express an antigen against which antibodies can be generated, there is no evidence that they are able to give rise to an immune response when administered orally. As discussed below, Figure 4 does not provide such evidence. Therefore, the subject matter of Applicants' claims is novel over Pouwels *et al.*

To emphasize this difference, independent claims 1, 18, 20 and 22 are amended to clarify that the vaccines of Applicants' invention elicit or induce an immune response when administered orally. Thus, the claims are not simply directed to something that is antigenic, but are directed to something that actually gives rise to an immune response when administered orally.

It was argued in the Office Action that because section 3.5 of Pouwels *et al.* refers to *Lactobacillus* which had been cultured for 7 hours, that it is apparent from Figure 4 that *Lactobacillus plantarum* ATCC 14917 was the strain cultured for 7 hours and hence was the strain administered. That is not the case, however, as all of the strains shown in Figure 4 were incubated for 7 hours and hence it is not possible to tell which strain was actually administered because Pouwels *et al.* only refers to adminis-

tration of *Lactobacillus* which could, and indeed was most likely to have been, *Lactobacillus casei*.

As indicated by Pouwels *et al.* at the paragraph bridging pages 188 and 189, Figure 4 depicts expression levels of a fusion protein in four different strains of *Lactobacillus*: *Lactobacillus plantarum* ATCC 8014, *Lactobacillus plantarum* ATCC 14917, *Lactobacillus* NCIB8826, and *Lactobacillus casei* ATCC393. Their expression levels were measured at each of four time-points: 6 hours, 7 hours, 8 hours, and overnight. Thus, the graph in Figure 4 shows four bars for each strain indicating the results for each of the four time-points (the bar for the overnight results shaded black is only just visible for some of the strains to indicate expression was very low).

Under the graph of Figure 4, a key is provided showing which type of shading corresponds to which time-point. Thus, the bar showing the shading for 7 hours being underneath the results for *Lactobacillus plantarum* ATCC 14917 is a coincidence, as it does not indicate that this was the only strain incubated for 7 hours. The interpretation of Figure 4 in the Office Action cannot be correct because, if it were, why are there four sets of bars for each strain in the graph? The paragraph bridging pages 188 and 189 discusses the "pattern" of expression for each strain which further demonstrates that all of the strains were assessed at all of the time-points.

In summary, all four of the strains shown in Figure 4 were incubated for 7 hours. Section 3.5 of Pouwels *et al.* only refers to "oral administration of *Lactobacillus* . . . cultivated for 7 h."

There is also no disclosure of administering all of the *Lactobacillus* strains and hence it is possible and, indeed, plausible that only one of the strains was administered. Any one of the strains could have been administered and one of skill in the art would have no way of knowing exactly which strain was in fact administered in Pouwels *et al.* In such a situation where it is simply not possible to tell which strain was administered, it is submitted that the cited reference cannot anticipate Applicants' claimed invention.

Although the strain administered in Pouwels *et al.* is not disclosed, logically, it would most likely be *Lactobacillus casei* 393. Figure 4 shows that *Lactobacillus casei* 393 was the strain giving the highest and most consistent expression of the fusion

protein over time. One of skill in the art would therefore have been most likely to have selected *Lactobacillus casei* 393 as that person would have considered protein expression levels to be an important factor in eliciting an immune response.

Given that oral vaccination was notoriously a difficult route to successfully elicit an immune response, the skilled person would have selected the strain they would have thought had the best chance of success, i.e., the one with the highest and most stable expression of the antigen namely *Lactobacillus casei* 393. Figure 1 also shows that *Lactobacillus casei* 393 achieves the best results for all of the strains in inducing DTH following intraperitoneal administration and hence making it is even more likely that the skilled person would have selected *Lactobacillus casei* 393 from the other strains when producing a vaccine for oral consumption.

It is therefore most likely that the strain *Lactobacillus casei* 393 was administered in Pouwels *et al.* Indeed, the use of *Lactobacillus plantarum* strains in oral vaccination as claimed by Applicants would have been judged counterintuitive from the disclosure of Pouwels *et al.* because *Lactobacillus plantarum* strains provided lower and less stable expression of the antigen than *Lactobacillus casei* 393. It is therefore surprising that *Lactobacillus plantarum* is much more effective at generating an immune response as illustrated by the stronger response when given intranasally as shown by Table 2A on page 33 of the present specification. Hence, it is unexpected that the bacterium *Lactobacillus plantarum* is able to give rise to an immune response whereas *Lactobacillus casei* does not.

There is no clear and unambiguous demonstration in Pouwels *et al.* of any *Lactobacillus plantarum* strain expressing a heterologous antigen which elicits or induces an immune response against the heterologous antigen when administered orally as recited in Applicants' claims. And it cannot be assumed, even given the "evidence" provided by the secondary references, that all of the strains discussed in Pouwels *et al.* would give rise to an immune response when administered orally. The subject matter of the claims is therefore novel over Pouwels *et al.*

Claims 20 and 22 were rejected under Section 102(b) as allegedly anticipated by Mercenier *et al.* (Adv. Food Sci. 18:73-77, 1996). Applicants traverse because claims 20

and 22 refer to *Lactobacillus plantarum* and there is no mention whatsoever of this bacterium in Mercenier *et al.* The subject matter of the claims is therefore novel over Mercenier *et al.*

Mercenier *et al.* are principally concerned with *Lactobacillus paracasei* (see abstract and section 2.3). *Lactobacillus paracasei* is the only bacterium that was administered to mice and for which an immune response was assayed. Other *Lactobacillus* species were assessed for their ability to colonize mice (see section 2.4) but these do not include *Lactobacillus plantarum.* In the absence of any disclosure of *Lactobacillus plantarum,* the subject matter of the claims is novel.

It is also highlighted in Mercenier *et al.* that the only immune response seen was against the M6 carrier protein and not the intended antigen. That is why M6 is called a carrier protein, it was not intended to induce an antigenic response. Figure 1 of Mercenier *et al.* shows that an immune response was seen against the M6 carrier protein. But Mecenier *et al.* state at page 75 that:

> None of the immunizations led to a detectable and specific anti-epitope antibody response, and no anti-V3 or anti-gp41E **APC**'s (**A**ntigen **P**resenting **C**ells) could be observed by ELISPOTs [emphasis added].

Applicants submit that M6 carrier protein does not represent an antigen. Whether or not M6 is an antigen, however, oral administration of the *Lactobacillus paracasei* species in Mercenier *et al.* completely failed to induce an immune response against the intended pathogenic antigen which was fused to M6.

There is no benefit in inducing an immune response against the carrier protein because it is not involved in the condition the subject is being vaccinated against (indeed it may be disadvantageous to do so, for instance if M6 is used as a carrier protein in a range of vaccines then the vaccinated individual would mount an immune response against the carrier protein every time they were vaccinated which is clearly undesirable). For instance, the gp41 antigen in the gp41-M6 fusion originates from HIV. Inducing an immune response against the M6 carrier will be of no benefit in vaccinating a subject against HIV. Mercenier *et al.* is not a promising starting point for someone hoping to develop an oral vaccine. Furthermore, it focuses on *Lactobacillus paracasei*

which is a much poorer species than *Lactobacillus plantarum* for generating an immune response.

Mercenier *et al.* employed *Lactobacillus paracasei* instead of *Lactobacillus plantarum*, and their vaccine failed to elicit an immune response against the intended pathogenic microorganism. The subject matter of the claims is therefore novel over Mercenier *et al.*

Claims 1 and 8 were rejected under Section 102(a) as allegedly anticipated by Maassen (J. Immunol. Meth. 223:131-136, 1999). Applicants traverse.

The subject matter of the claims is novel over the cited reference because there is no disclosure in Maassen of a *Lactobacillus plantarum* strain expressing a heterologous antigen that gives rise to an immune response when administered orally. As discussed above with respect to Pouwels *et al.*, it is fundamental when considering vaccines to remember that there is a substantial difference between antigenicity (i.e., something that antibodies can potentially be generated against) and immunogenicity (i.e., something that actually gives rise to an immune response).

Applicants' claims recite that *Lactobaccillus plantarum* gives rise to an immune response against its heterologous antigen when administered orally to emphasize the difference between immunogenicity and antigenicity. Thus, the ability to give rise to an immune response is not an optional feature and has to be taken into account when assessing the subject matter of the claims.

Although Maassen refers to bacteria which express antigens, there was no demonstration that any of them generated an immune response when administered orally. In the absence of any such evidence, Maassen cannot be said to disclose a *Lactobacillus plantarum* strain capable of inducing an immune response against a heterologous antigen expressed by the strain when administered orally. The subject matter of all of the claims is therefore novel over Maassen.

It was argued in the Office Action that Maassen refers to induction of antibodies following oral administration. Maassen does not, however, provide any experimental data showing that any of the *Lactobacillus plantarum* strains referred to induce an immune response. Section 2.3 of Maassen cited in the Office Action is concerned with

Western blotting of extracts from bacteria grown in culture using an antibody MBP or *Helicobacter pylori*. That is not the same as examining whether a vaccine elicits an immune response when administered orally.

Maassen was therefore simply analyzing whether a protein is expressed *in vitro*; it was not examining whether an immune response is induced following oral administration. That is apparent from section 2.3 which states that:

> To induce expression, cells were grown in modified MRS medium (mMRS) . . . Cells were harvested in exponential phase, pelleted by centrifugation and suspended in phosphate buffered saline (PBS). Cell extracts were obtained . . . . After centrifugation the soluble fraction was used for immunoblot analysis.

It is thus clear that the bacteria were not administered to mice and no analysis of any immune response was carried out. There is no demonstration that any of the species discussed in Maassen can give rise to an immune response when administered orally. In the field of oral vaccines, which is littered with vaccines that proved to be ineffective failing to give rise to an immune response, this is an important distinction.

Thus, there is no evidence that any of the species in Maassen would give rise to an immune response when administered orally. In the absence of any such disclosure, the subject matter of the claims is novel over Maassen.

Claims 1-6, 9-12, 14-15, 20-21, 24 and 31-32 were rejected under Section 102(b) as allegedly anticipated by Madsen *et al.* (WO 98/10079). Applicants traverse.

There is no disclosure in Madsen *et al.* of any *Lactobacillus plantarum* strain that expresses a heterologous antigen which will give rise to an immune response against the heterologous antigen when administered orally. As discussed above, in the absence of any evidence that a bacterium gives rise to such an immune response when administered orally, the subject matter of the claims is novel over the cited reference. It cannot be assumed that strains would inherently have such properties.

Madsen *et al.* are concerned with the use of promoters which are regulatable by various environmental factors to express proteins in *Lactobacillus* (see page 8, lines 17 to 23). As highlighted at page 3, lines 28-30, inducible systems can be used, inter alia, to express gene products that are toxic to the host organism. Madsen *et al.* also refer to

the possibility of expressing "immunologically active compounds" using such promoters. There is no evidence, however, that any of the bacteria described by Madsen *et al.* would be able to give rise to an immune response when administered orally as required by Applicants' claims.

In particular, none of the examples of Madsen *et al.* provided any immunological data and in addition they focus on *Lactobacillus lactis*. The examples principally deal with the characterization of the p170 pH regulated promoter of *Lactobacillus lactis* and its use in various constructs. Example 11 of Madsen *et al.* deals with the use of such promoter to express "two *Mycobacterium tuberculosis* antigens, MPT64 and ESAT-6." The bacteria produced by Madsen *et al.*, however, are not orally administered to any organism so there is no experimental data showing whether or not the strains are capable of giving rise to an immune response. Madsen *et al.* refer to Western blotting (see page 70) but that is simply to monitor protein expression, not to look for an immune response in a vaccinated subject.

Thus, there is no demonstration that any of the bacteria disclosed in Madsen *et al.* were able to give rise to an immune response when administered orally. Moreover, the examples did not provide any evidence that the specific *Lactobacillus lactis* strain generated expressing MPT64 or ESAT-6 can give rise to an immune response, let alone any *Lactobacillus plantarum* strain can give rise to an immune response when administered orally. It is highlighted that Madsen *et al.* refers to *Lactobacillus plantarum* only once; the focus of Madsen *et al.* is on other *Lactobacillus* species and, in particular, *Lactobacillus lactis.*

As discussed above, the distinction between a substance being antigenic and immunogenic is fundamental. Just because Madsen *et al.* indicate that antigens can be encoded by the various constructs does not mean that the bacteria referred to will give rise to an immune response.

In the absence of any experimental data in Madsen *et al.* demonstrating that an immune response has been elicited and, in particular, of evidence of eliciting or inducing an immune response after oral administration of *Lactobacillus plantarum* expressing a heterologous antigen, it cannot be said that a *Lactobacillus plantarum* strain which gives

rise to an immune response following oral administration has been disclosed either explicitly or inherently. The subject matter of the claims is therefore novel over Madsen *et al.* for the same reasons that it is novel over the other cited references.

It is also highlighted that Madsen *et al.* does not discuss the use of *Lactobacillus* to express "immunologically active gene products" for oral administration or that they should be formulated in a form suitable for oral administration. As highlighted in the Office Action, Madsen *et al.* refer to foodstuffs at a number of places including page 7, last paragraph. Madsen *et al.* also refers to probiotically active bacteria at pages 18 and 19, but the bacteria could well be acting by competition rather than via an immune response. There is no discussion of eliciting or inducing an immune response in this section.

In the section of Madsen *et al.* specifically discussing immunologically active compounds at page 20, lines 5-31, there is no discussion of oral administration and there is no section anywhere else in Madsen *et al.* discussing administration which refers to oral administration of bacteria encoding such "immunologically active compounds" as opposed to discussion of oral consumption of foodstuffs or probiotic bacteria. Oral administration of a lactic acid bacteria designed for foodstuffs or even probiotic lactic acid bacteria is not the same as oral administration of a vaccine, or formulation of bacteria in a form suitable for oral administration, where the bacteria are *Lactobacillus plantarum* expressing a heterologous antigen.

The subject matter of the claims is therefore novel over Madsen *et al.* both because there is no evidence of a *Lactobacillus plantarum* strain expressing a heterologous antigen which gives an immune response when administered orally and also because there is no discussion of oral administration, or a formulation suitable for such administration, when *Lactobacillus plantarum* expresses a heterologous antigen.

Withdrawal of the Section 102 rejections is requested because all limitations of the claimed invention are not disclosed by the cited references.

Conclusion

Having fully responded to all of the pending objections and rejections contained in this Office Action, Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

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